MICROWAVE THAWING OF FROZEN PACKED RED BLOOD CELLS

N. L. Campbell
Naval Ocean Systems Center
Bioengineering Branch
San Diego CA 92152

J. Drewe
San Diego State University Foundation
San Diego CA 92182

ABSTRACT

Small samples of frozen human packed red blood cells (RBCs) were thawed by microwave heating (2450 MHz) and by water bath heating. The functional integrity of the cells was measured before and after thaw, and over a 6-hour period after wash. Minor differences between microwave and water thawed RBCs were observed.

Introduction

At present, the accepted method of thawing frozen packed red blood cells (RBCs) is in a warm water bath. This thawing method introduces bacterial contamination in approximately 2-5% of the units (1,2,3). Because of this potential for contamination, RBC units must be transfused within 24 hours of thaw, or be discarded. Elimination of the contact of the blood unit with the water of the bath could extend this 24-hour post thaw period to 72 hours or more, thereby decreasing the waste of thawed blood and increasing the efficiency of the frozen blood program. Currently, many blood banks are double bagging their frozen units before placing in the water bath. Another method of aseptic thawing, perhaps more convenient than double bagging, is the use of the dry heating of microwave energy. Acceptability of a microwave thawing system requires (1) elimination of the possibility of overheating which causes cell damage, and (2) assurance that if overheating is eliminated, the cells are not damaged by some other characteristic of microwave heating. Attempts are currently underway in our laboratory to develop a microwave thawing system which eliminates the possibility of overheating the blood. The latter problem of potential damage not associated with overheating has been the basis of recent investigation in our laboratory, and is the topic of this paper.

A potential destructive effect of microwave energy is denaturation of proteins. Altering the structure of many of the RBC proteins could adversely affect their structural or metabolic functions, thereby decreasing the RBC's value in transfusion therapy. The purpose of this study was to determine the feasibility of using microwave energy to thaw full units of RBCs by comparing the in vitro characterististics of small samples of microwave thawed RBCs (MT-RBCs) to the in vitro characteristics of small samples of water bath thawed RBCs (WT-RBCs). Consistent differences in RBC characteristics between MT- and WT-RBCs would reflect any effects due to the thaw method, and indicate potential hazards of the microwave thawing process. To determine consistent differences, samples of frozen RBCs were thawed using both methods and analyzed for RBC characteristics. The data were then averaged and a two-sample comparison of means test performed.

Procedure

Viability Tests

The parameters measured included hematocrit (the volume percent of RBCs in a solution), RBC count, pH level, and the concentrations of hemoglobin (Hb), glucose, lactate, 2,3-diphosphoglycerate (DPG), adenosine triphosphate (ATP) and reduced glutathione (GSH). The latter three biochemicals are by-products of the cell's metabolism and are necessary for proper function of the hemoglobin. In addition to these measurements, calculations were made for percent recovery (the percentage of cells that survive a particular procedure, abbreviated as %R), percent hemolysis (the percentage of broken cells in a sample at any one time, abbreviated as %H), mean cellular hemoglobin concentration (MCHC), mean cellular hemoglobin (MCH), mean cellular volume (MCV), and the rate of cellular metabolism as represented by the rates of glucose decrease and end product (lactate) increase, expressed as μmol/gm Hb-min. These rates were calculated for each 2-hour period post wash (0 to 2 hours, 2 to 4 hours, and 4 to 6 hours), and for the overall period of incubation (0 to 6 hours).

Preparation

Units of human RBCs were prepared for freezing using glycerolization and rejuvenation techniques developed by Dr. C. R. Valeri of the Naval Blood Research Laboratory, Boston, MA. Dr. Valeri's procedure for glycerolization includes removal of excess glycerol before freezing to concentrate the glycerolized cells to a hematocrit of about 60%. Two units were obtained fresh and required no rejuvenation. Six units were obtained outdated and were rejuvenated. Morphologic and metabolic parameters were measured, and then multiple samples (3.5 ml) of each unit were frozen in a mechanical freezer at -75°C for at least 24 hours.

Thawing

Half of the samples from each unit were thawed in a warm water bath at 42°C for 5 minutes (previously determined to give optimal yields), the other half using microwave energy at 2450 MHz. This frequency was chosen because of possible future adaptation of a commercial microwave oven for full unit thaws. A tube containing the frozen sample was inserted into a traveling waveguide (see Figure 1); power was turned on at 300 W and reflected power was monitored. The sample was constantly rotated in the waveguide by manual turning to minimize hot spot development. Reflected power began to rise from the initial tuned setting of zero after about 30 seconds, indicating the initiation of phase change in parts of the sample. Power was turned off when the reflected power reached 20 W. The power was then pulsed at approximate intervals of 2 seconds on, 10 seconds off, until the entire sample was thawed as indicated by a free flowing liquid when the tube was tilted.

0149-645X/81/0000-0479$00.75

Post Thaw Studies

Total hemoglobin, supernatant hemoglobin, and hematocrit were measured after thaw to calculate percent recovery, percent hemolysis, and MCHC. The cells were then aseptically washed of the glycerol by repeated NaCl dilution, supplied with glucose and buffer (4), and placed in a 37°C water bath for 6 hours of incubation. Both morphologic and metabolic parameters were measured immediately after wash (0 hour) and at 2, 4, and 6 hours after wash. Microwave thawed and water bath thawed samples from the same original unit of blood were washed and tested side by side to minimize potential variables other than the thawing procedure.

Results

Fifty experiments were performed using the microwave thawing method, 51 using the water bath thawing method. The data were pooled per thawing technique, averages were calculated, and a two-sample comparison of means test was performed. This statistical test was performed without setting a predetermined confidence level (i.e., without setting an α value). Instead, confidence levels of significance were calculated for each Z-statistic. The number of samples examined from each unit ranged from three to nine pairs of thaws. Table 1 summarizes the total sample size (n), average ($\bar{x}$), standard deviation (s), and Z-statistic for most of the parameters measured.

Post Thaw

MT-RBCs averaged a slightly higher freeze-thaw percent recovery (97.1%) compared to WT-RBCs (96.4%) with a calculated confidence level of significance of 94% (i.e., α was calculated to be 0.06). This higher percent recovery was supported by a slightly lower percent hemolysis in MT-RBCs (3.9%) compared to WT-RBCs (4.6%), with a calculated confidence level of significance of 94%. No significant differences were observed in total hemoglobin, supernatant hemoglobin, hematocrit, or MCHC immediately after thaw.

Post Wash

The wash percent recovery showed no significant difference between MT- and WT-RBCs. No significant differences were observed throughout the 6 hours of post wash incubation in hemoglobin, percent hemolysis, hematocrit, RBC count, MCHC, MCH, or MCV. The results of the metabolic study are discussed below.

Figures 1 and 2 show the change in the Z-statistic (representing differences between MT- and WT-RBCs) over the 6 hours post wash. A positive Z indicates that the MT average is greater than the WT average, and a negative Z indicates that the MT average is less than the WT average. A Z value of zero indicates no difference. Calculated confidence levels of significance are indicated for the more significant differences (confidence levels greater than 90%). Though lines are drawn connecting points for easier viewing, data were collected only at 0, 2, 4 and 6 hours post wash.

Figure 1 shows the difference in glucose concentration between MT- and WT-RBCs over time. As can be seen, the difference in glucose concentration jumps significantly at 6 hours post wash (confidence level of 95%), MT-RBC samples having higher glucose concentrations than WT-RBC samples at these times. This onset of a difference at 6 hours indicates that MT-RBC metabolism slows down compared to WT-RBC metab-

TABLE 1: SUMMARY OF MT- AND WT-RBC CHARACTERISTICS

PARAMETER	MT-RBC			WT-RBC			Z
	n	$\bar{x}$	s	n	$\bar{x}$	s	
Percent Recovery							
Freeze-Thaw	50	97.1	1.7	51	96.4	2.5	1.6
Wash	47	79.7	11.6	47	80.6	11.2	-0.4
Percent Hemolysis							
Freeze-Thaw	50	3.9	1.7	51	4.6	2.5	-1.6
Hour 0	47	1.0	0.9	47	0.9	0.7	0.3
Hour 2	47	1.0	0.8	47	1.1	1.0	-0.3
Hour 4	46	1.1	0.7	46	1.2	0.9	-0.2
Hour 6	46	1.5	0.8	46	1.5	0.8	-0.3
MCHC							
Freeze-Thaw	50	31.9	1.7	51	31.5	1.7	1.2
Hour 0	47	38.3	2.6	47	38.2	2.7	0.2
Hour 2	47	38.4	2.6	47	38.1	2.4	0.6
Hour 4	46	38.3	2.7	46	37.9	2.5	0.8
Hour 6	46	38.1	2.9	46	38.0	2.7	0.2
MCV							
Hour 0	44	77.3	6.3	46	78.2	6.1	-0.7
Hour 2	41	76.2	5.2	41	77.0	4.5	-0.7
Hour 4	40	76.9	4.7	40	77.3	4.4	-0.4
Hour 6	40	76.9	4.5	40	76.7	4.4	0.2
MCH							
Hour 0	44	29.6	1.8	46	29.8	1.7	-0.5
Hour 2	41	29.4	1.5	41	29.5	1.4	-0.2
Hour 4	40	29.0	4.6	40	29.5	1.6	-0.6
Hour 6	40	29.5	1.8	40	29.3	1.7	0.4
DPG (µmol/gm Hb)							
Hour 0	47	17.6	5.8	47	16.9	6.1	0.6
Hour 2	47	18.0	6.9	47	17.8	6.8	0.1
Hour 4	46	16.6	7.3	46	16.8	7.3	-0.2
Hour 6	46	14.4	8.9	46	14.4	7.7	0.0
ATP (µmoles/gm Hb)							
Hour 0	46	6.0	1.5	47	5.9	1.4	0.2
Hour 2	46	5.8	1.2	46	5.8	1.2	-0.2
Hour 4	45	5.7	1.5	45	5.8	1.3	-0.4
Hour 6	44	5.7	1.2	44	5.7	1.4	0.1

PARAMETER	MT-RBC			WT-RBC			Z
	n	$\bar{x}$	s	n	$\bar{x}$	s	
GSH (µmoles/gm Hb)							
Hour 0	29	6.0	2.5	29	5.9	2.5	0.1
Hour 2	29	6.1	2.5	29	6.1	2.7	0.1
Hour 4	29	6.0	2.3	29	6.0	2.3	-0.1
Hour 6	27	6.0	2.5	28	5.9	2.3	0.1
Glucose (µmol/gm Hb)							
Hour 0	47	77.4	20.0	46	75.1	20.0	0.6
Hour 2	47	65.6	19.7	47	64.1	19.3	0.4
Hour 4	46	58.8	22.8	46	56.4	19.4	0.5
Hour 6	45	50.0	20.9	45	43.0	18.9	1.7
Glucose rate decrease (µmol/gm Hb-min)÷100							
Hour 0-2	47	9.8	6.9	46	8.8	6.4	0.7
Hour 2-4	46	6.1	7.8	46	6.8	5.2	-0.5
Hour 4-6	46	7.1	4.0	46	6.9	5.0	0.2
Hour 0-6	45	7.8	1.8	44	7.6	2.0	0.5
Lactate (µmol/gm Hb)							
Hour 0	41	17.0	9.8	41	15.4	8.9	0.8
Hour 2	41	39.0	10.2	41	38.0	9.7	0.4
Hour 4	40	57.8	10.7	40	57.2	10.8	0.3
Hour 6	40	74.5	12.3	40	75.9	11.9	-0.5
Lactate rate increase (µmol/gm Hb-min)÷100							
Hour 0-2	41	18.3	3.6	41	18.9	3.1	-0.8
Hour 2-4	40	15.8	3.9	40	16.1	3.7	-0.4
Hour 4-6	40	13.9	5.1	40	15.5	5.0	-1.4
Hour 0-6	40	16.0	2.5	40	16.8	2.3	-1.6
pH							
Hour 0	44	7.5	0.1	44	7.5	0.1	0.0
Hour 2	45	7.4	0.1	45	7.4	0.1	0.1
Hour 4	44	7.3	0.1	44	7.3	0.1	0.6
Hour 6	38	7.2	0.1	40	7.2	0.1	0.2

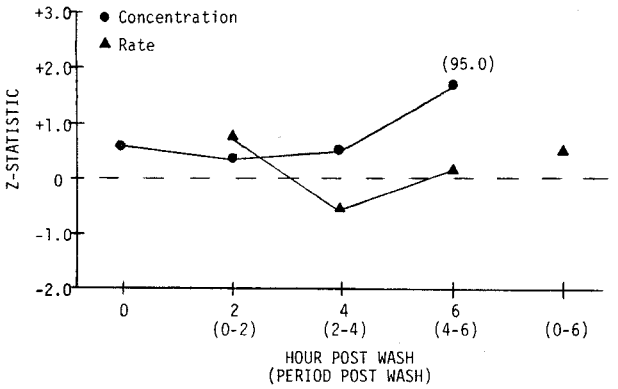

FIGURE 1: GLUCOSE CONCENTRATION AND GLUCOSE RATE OF DECREASE OVER TIME. (THE Z-STATISTIC REPRESENTS THE DIFFERENCE BETWEEN MT- AND WT-RBCs.)

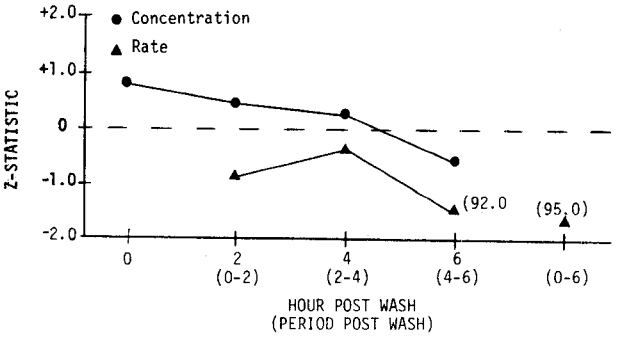

FIGURE 2: LACTATE CONCENTRATION AND LACTATE RATE OF INCREASE OVER TIME. (THE Z-STATISTIC REPRESENTS THE DIFFERENCE BETWEEN MT- AND WT-RBCs.)

olism. However, a significant difference in metabolic rate is not indicated from the measured rate of glucose decrease, also represented in Figure 1. The rate of glucose decrease was calculated for each 2-hour period and for the overall 6-hour period studied. The periods are indicated in parentheses on the graph.

Figure 2 shows the difference between MT- and WT-RBC lactate concentrations over time, and the difference in the rate of lactate increase. No significant differences were observed in lactate concentration over time. However, significant differences were observed in the rate of lactate production between 4 and 6 hours, and overall between 0 and 6 hours post wash. The MT-RBC rates of lactate increase were slower than the WT-RBC rates at these times, again suggesting a slowdown in MT-RBC metabolism. No significant differences were observed in concentrations of DPG, ATP or GSH over the 6 hours post wash.

Discussion

The freeze-thaw percent recoveries averaged around 97%, barely meeting the minimum freeze thaw percent recovery acceptable in blood banks. This low average percent recovery may be due to the use of outdated-rejuvenated RBCs in this study. The percent recovery from the two units of fresh RBCs used averaged 99%. Also, this low percent recovery may be due to the use of very small aliquots (3.5 ml) of frozen RBCs compared to the large units used in blood banks. A difference in the average percent recoveries of the two methods of thawing indicates that microwave thawing is not lysing the cells more than water bath thawing, and may even yield greater percent recoveries.

The average wash percent recovery from both methods of thaw was about 80%. This low recovery is probably due to the manual washing techniques used in this study versus the automated washing techniques used in a blood bank. No difference between the two thawing methods was observed in this study in wash percent recoveries. However, this parameter will be remeasured in future studies following automated washing of both MT- and WT-RBCs.

Possible hazards of microwave thawing were observed in metabolic rates. MT-RBCs may metabolize more slowly than WT-RBCs, as indicated by a significant difference in the average glucose concentration at Hour 6 post wash, and by significant differences in lactate increase between Hours 4 and 6, and overall between Hours 0 and 6. However, these differences may not be conclusive since no differences were observed in the supporting data of the rate of glucose decrease, and the lactate concentrations. More studies should be undertaken to verify a difference in metabolic rate. A slower metabolic rate in MT-RBC may or may not be hazardous. Harm would be indicated only if this slowdown caused either a shortened *in vivo* life of the cell, or a drop in the production of functional by-products. As seen in this study, the production of the important by-products of metabolism (ATP, DPG, GSH) was not affected by microwave thawing, at least over 6 hours post wash.

In conclusion, microwave thawing does not seem to damage RBCs as long as the blood is not overheated. The possibly slower metabolic rate induced by microwave thawing should first be substantiated and, if necessary, potential repercussions analyzed.

REFERENCES

1. Meryman, H.T., and R.A. Kahn. 1976. Bacteriological quality of frozen red cells 72 hours following deglycerolization, in Sherer, P.B. (ed.), *Frozen red cell outdating*. U.S. Dept. of Health, Education and Welfare, DHEW Publication No. (NIH) 76-1004.

2. Szymanski, I.O., and E.J. Carrington. 1977. Evaluation of a large-scale frozen blood program. Transfusion 17(5):431-437.

3. U.S. Department of Health, Education and Welfare. 1979. Nosocomial *Pseudomonas cepacia* infection. Morbidity and Mortality Weekly Report 28(25):289-290.

4. Campbell, N.L. 1979. *In vitro* viability studies of microwave thawed red blood cells. Masters Thesis, San Diego State University.